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SCLEROTINIA RESISTANCE AND IDENTIFICATION OF MOLECULAR MARKERS FOR CANADIAN CANOLA CULTIVARS

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Development of *Brassica napus* germplasm with sclerotinia resistance derived from a Chinese cultivar Zhongyou 821, and identification of molecular markers for accelerated breeding of Canadian canola cultivars.

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March 2003 to June 2006.

(a) Abstract

At AAFC, Saskatoon Research Centre we have utilized resistance to sclerotinia stem rot (*Sclerotinia Sclerotiorum*) in the Chinese *B. napus* winter-type cultivar Zhong You 821 (ZY821) for mapping and marker development (this project), cultivar development (Drs. Seguin-Swartz and Rakow), and resistance gene identification (Drs. Hegedus, Rimmer and Buchwaldt). Progress in the three areas relies on a sclerotinia screening method developed in the present project, in which differentiation between resistant and susceptible lines can be obtained by inoculating stems with a fixed amount of mycelium harbouring a known level of plant cell-wall degrading enzymes. Initially, three populations of double haploid (DH) lines were developed from Bao604 x ZY821 (DH1), DH6756 x ZY821 (DH2), and DHWestar x ZY821 (DH3). To increase the population size and avoid the winter-type trait new populations were developed from two backcrosses of the most resistant spring-type DH lines to DHWestar (DH4 and DH5). About 700 microsatellite markers were selected potentially amplifying more than 900 loci covering the 19 *B. napus* linkage groups. About 50% of the markers were polymorphic between ZY821, Westar and Bao604. However, polymorphism between ZY821 and DH6756 was low, and the DH2 population was omitted from the study. Linkage maps of microsatellite markers for each of the five remaining DH populations were developed. Reliable phenotypic disease data were obtained from 59 DH1-117 lines, 63 DH3 lines, 146 DH4 lines and 30 DH5 lines encompassing 28,800 data points (i.e. 300 lines x app. 8 plants/line x 2 inoculation sites/plant x 6 observations/inoculation site). QTL analysis of the data from DH4 resulted in four QTLs on linkage groups N6, N7, N11 (LOD = 3.60, 1.3 cM) and N16 (LOD = 3.3, 16.8 cM). Screening of DH3 yielded six QTLs on linkage groups N3, N5 (LOD = 2.71, 2.9 cM), N16 and N19 (LOD = 2.78, 2.78 cM), and DH1-117 resulted in three QTLs on N9 (LOD = 2.66, 1.2 cM), N11 and N12, respectively. The variances explained by each sclerotinia trait ranged from 11.4 % to 19.4%. The lack of corresponding QTLs among populations is thought to be due in part to small population sizes and to differences in the genetic contribution from the susceptible parents, Bao604 and DHWestar, affecting stem thickness, plant height and vernalization requirements. F_1 progeny from crosses between the most resistant DH lines and DHWestar were susceptible showing resistance to stem rot is recessive. Some F_1 progeny from crosses among resistant and moderately resistant DH lines were highly resistant indicating that important QTLs were shared between the parental lines resulting in homogeneity; while other F1 progeny were susceptible probably as a result of heterogeneity at certain QTLs.

We are encouraged by the above results and believe no other research group can match the progress we have made in transferring sclerotinia resistance to canola and mapping of loci conferring resistance to stem rot. In the next phase of the project recombinant inbred lines (RILs) will be developed since more recombinations will occur around the putative sclerotinia QTLs than in DH lines. Certain RI lines will have a combination of homogeneity and heterogeneity at the QTLs we are interested in, and phenotyping of selfed progeny from these RI lines will narrow the QTLs to those with significant effect on resistance and allow identification of closely linked markers. We have submitted a letter of intent to ADF in April 2006 for co-funding of the second phase of this project.

(b) Executive summary

Resistance to sclerotinia stem rot in canola is a highly desirable but rare trait. We have utilized sclerotinia resistance from *B. napus* cultivar Zhong You 821 (ZY821) a winter-type cultivar developed at the Oilseed Crops Research Institute in Wuhan, China. The objectives were to transfer stem rot resistance into spring-type *B. napus* and identify microsatellite markers linked to sclerotinia resistance for use in canola breeding programs. The resistant ZY821 was crossed with susceptible parents and populations of doubled haploid (DH) lines developed. These DH populations are suitable for marker development and mapping of quantitative trait loci (QTL), since some lines harbour resistance genes in a homozygous state while other lines have none.

The major accomplishments achieved in the project

A reliable sclerotinia screening method was developed

It was demonstrated that the sclerotinia phenotyping method that was developed resulted in reproducible disease severities by inoculation of selected resistant and susceptible DH lines at four separate test dates (Figure 1). There was a three to four fold difference between resistant and susceptible DH lines. The sclerotinia phenotyping methodology was presented orally or as posters on several occasions (see publication list), and ADF's financial contribution was acknowledged. A scientific paper describing the screening method is in preparation.

Seed of five populations of doubled haploid lines segregating for resistance to sclerotinia stem rot are maintained at AAFC, Saskatoon

We have developed five populations of double haploid *Brassica napus* lines segregating for resistance to sclerotinia stem rot. Selfed seed are available of 80 DH1-117 lines, 74 DH1-118 lines, 66 DH3 lines, 234 DH4 lines and 186 DH5 lines. The seed have been increased and are stored at AAFC, Saskatoon.

Sclerotinia phenotypic data were obtained from 300 DH lines

Reliable phenotypic disease data were obtained from 59 DH1-117 lines, 63 DH3 lines, 146 DH4 lines and 30 DH5 lines encompassing 28,800 data points (i.e. 300 lines x app. 8 plants/line x 2 inoculation sites/plant x 6 observations/inoculation site). Fourteen DH lines with sclerotinia stem rot resistance were identified, thereby demonstrating that sclerotinia resistance is heritable and can be transferred to canola cultivars. Seed of sclerotinia resistant spring-type DH lines are currently utilized for cultivar development and elucidation of the molecular interaction between the host and pathogen.

Linkage maps of putative QTLs for sclerotinia resistance were developed

Two linkage maps were developed of ZY821 x Bao 604 with 192 and 189 microsatellite loci, respectively (Figure 2 and 3). A linkage map was developed of ZY821 x Westar with 209 loci (Figure 4) and two maps with 94 and 83 loci were developed from backcrosses of the two most sclerotinia resistant spring –type lines to DH Westar (Figure 5 and 6). The sclerotinia phenotyping

data (described in item 3) was used for mapping of putative QTLs. Candidate QTLs with LOD scored above 2.5 were identified and are most likely to contribute to sclerotinia resistance (Table 2).

The dominance of resistance to S. sclerotiorum in B. napus was determined

Crosses were made between resistant and susceptible DH lines (R x S) and among resistant (R x R) DH lines (Table 3). Inoculation with sclerotinia of the F_1 progeny derived from S x R were susceptible (Figure 7) showing that resistance to stem rot is recessive. F_1 plants derived from R x R and R x MR crosses varied from highly resistant to almost as susceptible as DH Westar. Assuming stem rot resistance is recessive, the most resistant F_1 plants were likely derived from crosses between two DH lines sharing QTLs most responsible for the resistance expression. Currently, there are too may putative QTLs (Table 2) to allow conclusions on the effect of each individual QTL

Technologies transferred

1) Seed of spring-type *B. napus* with high level of resistance to *S. sclerotiorum* are included in the publicly accessible germplasm collection at PGRC, Saskatoon (Plant Gene Resources of Canada).

2) A stem rot resistant spring-type DH line was transferred to the canola breeding program at AAFC, Saskatoon (Drs. G. Rakow and G. Seguin-Swartz, Crop Improvement Group) where a cross was made with a high yielding, yellow-seeded canola line to develop the first sclerotinia resistant canola cultivar for Canada.

3) Information on our sclerotinia screening protocol was also transferred to Dr. Seguin-Swartz, and help was provided with screening of the first 300 F_2 plants for stem rot resistance.

4) Five resistant DH lines and five susceptible DH lines were transferred to Dr. D. Hegedus (Molecular Plant Protection Group) to help identify genes that are up or down regulated in stem tissues infected by *S. sclerotiorum* using a newly developed micro-array platform at AAFC; candidate stem rot resistance genes will be over-expressed in susceptible lines, or defence genes will be knock-out in resistant lines to determine their relative importance. Most importantly, it will be determined whether some of these defence genes correspond to the QTLs we have identified in the present study.

5) Results from our research on sclerotinia phenotyping and development of molecular markers linked to resistance QTLs were presented at 8 meetings from 2003 to 2005 as listed under publications on page 18.

6) Private and public canola breeders were informed about our project results and invited, non-exclusively; to contribute to further development of more closely linked sclerotinia markers in spring- and winter-type *B. napus*. A Collaborative Research and Development Agreement was signed between AAFC, SRC and four companies in 2005.

(c) Technical Report

A reliable sclerotinia screening method for B. napus

Clone #321 of *Sclerotinia sclerotiorum* was selected as inoculum due to its wide distribution in canola fields in western Canada (Kohli et al., 1995, Molecular Ecology 4: 69-77). To maintain aggressiveness of clone #321, mycelium was re-isolated from inoculated Westar plants every 6 months and stored at -80°C. To produce mycelium for inoculation, samples of the stored mycelium was plated on a glucose-rich agar medium modified from Cruickshank (1983, Trans. Br. Mycol. Soc. 80:117-119). This media was selected after comparison of several media resulting in different hyphal densities and various levels of cell-wall degrading enzymes, such as polygalacturonase (PG). Mycelium grown on the selected glucose-rich media had a low level of constitutively expressed PG1 in the growing margin and about 16 hyphae per mm (3 times less than PDA and V8-juice agar); most importantly, inoculation with this mycelium resulted in good differentiation between the resistant parent ZY821 and the two susceptible parents Westar and Bao604.

Plants were inoculated at full flower with 5 mm mycelium-plugs which were attached to the main stem with Parafilm, and prevented the mycelium from drying out. Each plant was inoculated at two mid-stem internodes with one un-inoculated internode in between. Five to 15 plants of each doubled haploid line were inoculated. The two parents were included as susceptible and resistant controls, respectively. Stem lesions lengths were measured 7, 14 and 21 days after inoculation (dai), and the texture of each lesion was recorded as either firm, soft or collapsed. The following variables were calculated for each line: the mean lesion length 7, 14 and 21 dai, the standard error of the mean, area under the disease progress curve (AUDPC), and percent soft plus collapsed lesions (% s + c) recorded over the three rating dates.

Statistical analysis showed there was a high positive correlation between lesion length 21 dai and % s + c (R = 0.80-0.84). There was a two to three fold difference between sclerotinia symptoms of the resistant and susceptible parents. The resistant parent, ZY821, had a mean lesion length of 50 +/- 6 mm 21 days after inoculation and less than 25% soft + collapsed lesions, while the susceptible parents, Westar and Bao604, had 130 -155 mm lesion lengths and 70 - 80% soft + collapsed lesions. A clear cut-off between resistant and susceptible DH progeny was not evident, but ten DH1 lines and four DH3 lines were consistently as resistant as ZY821.

To demonstrate reproducibility of the sclerotinia phenotyping method, resistant, moderately susceptible, and susceptible double haploid lines from the DH1, DH2 and DH3 populations were selected for screening at four separate dates. The result showed that 82 % of the lines behaved similarly over all test dates (Figure 1).

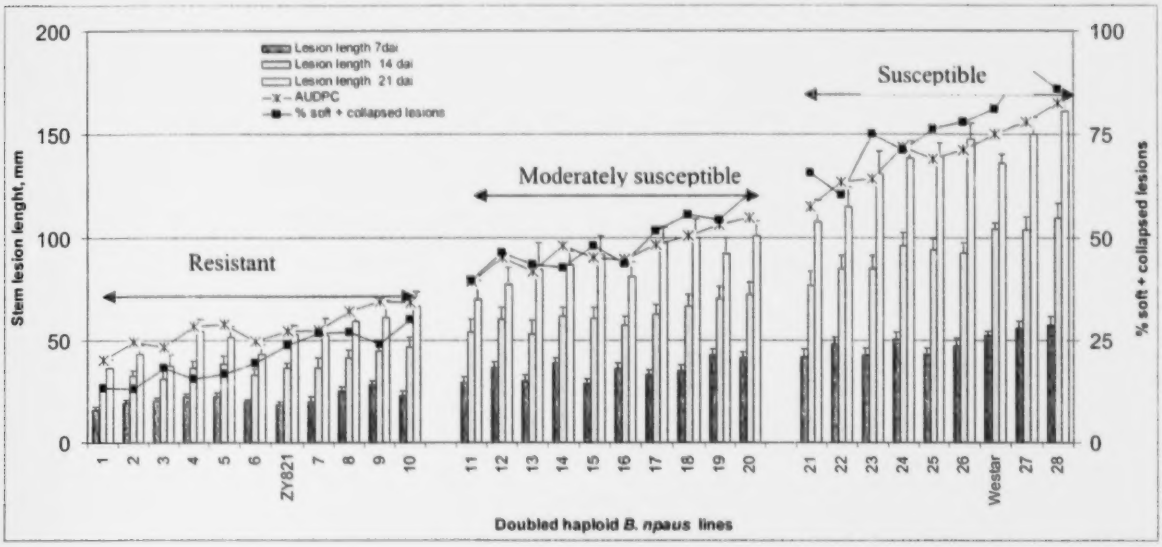

Figure 1. Disease reaction of selected resistant (1-11) moderately susceptible (11-20) and susceptible (21-28) lines from the DH1, DH2 and DH3 populations inoculated with *S. sclerotiorum* in four replicated test. Bartlett's tests for homogeneity of variances showed 82% of the lines were homogeneous over test dates. There was a two to three fold difference between resistant and susceptible DH lines, represented by ZY 821 and Westar, respectively.

Mapping of QTLs conferring resistance to sclerotinia stem rot in B. napus

Plant populations: Populations of doubled haploid (DH) lines were derived from the sclerotinia resistant cultivar Zhong You 821 (ZY821) from China (Figure 2). Initially ZY821 was crossed with three susceptible lines Westar, Bao604 and DH6756 and DH lines were obtained by colchicine treatment of F1 microspore cultures. A total of 80 (DH1-117) and 74 (DH1-118) lines were derived from Bao604 x ZY821; these were all winter types and needed 4 weeks vernalization (4 weeks at 5°C) to induce flowering. 78 (DH2) lines were derived from DH6756 x ZY821 and 66 (DH3) lines were derived from Westar x ZY821 both populations segregated for spring and winter types. To increase the population size and avoid the winter-type trait, two new populations were developed from crosses of a resistant spring-type DH3 line with DH Westar. Hybridisation was confirmed with polymorphic microsatellite markers, and F_1 plants were transferred to Dr. A. Ferrie at PBI, NRC for development doubled haploids using colchicine treatment of microspore cultures resulting in 234 DH4 and 186 DH5 lines of normal growth habit and seed set (Figure 2). 486 lines were discarded due to no or poor seed set most likely caused by lack of chromosome doubling.

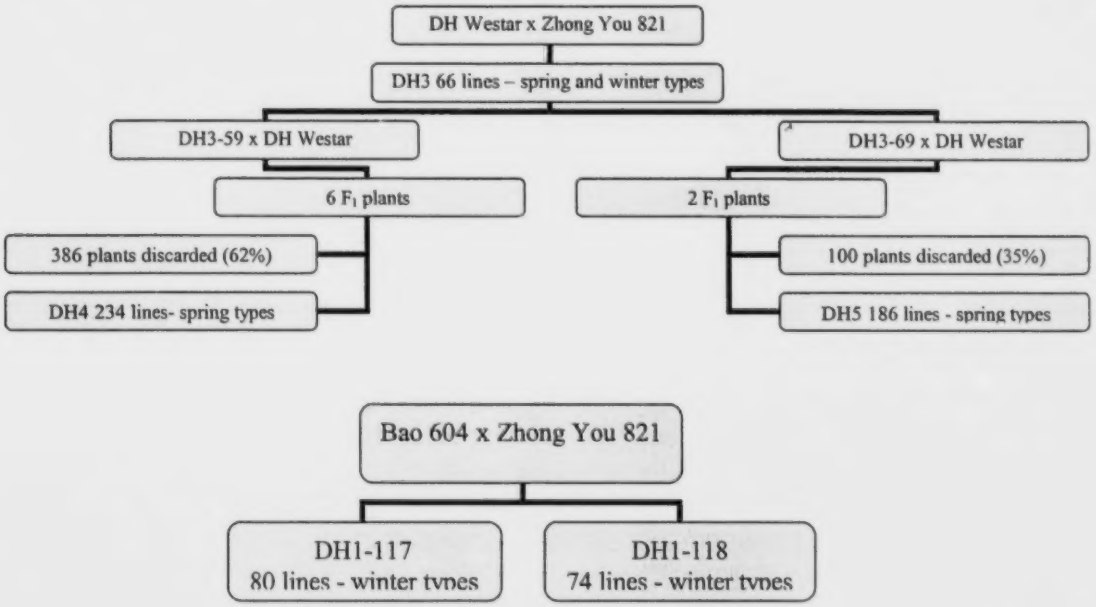
DH Westar x Zhong You 821
DH3 66 lines – spring and winter types
DH3-59 x DH Westar
DH3-69 x DH Westar
6 F1 plants
2 F1 plants
386 plants discarded (62%)
100 plants discarded (35%)
DH4 234 lines- spring types
DH5 186 lines - spring types
Bao 604 x Zhong You 821
DH1-117
80 lines - winter types
DH1-118
74 lines - winter types

Figure 2. Five doubled haploid populations were developed from a sclerotinia resistant cultivar Zhong You 821 for mapping of quantitative trait loci (QTLs) conferring resistance to this disease. One population of doubled haploid lines was derived from a cross between ZY821 and the Canadian spring type cultivar Westar. Two larger DH populations were developed from a backcross of the two most resistant spring type doubled haploid lines, DH3-59 and DH3-69, with DH Westar. ZY821 was also crossed with a winter type Chinese cultivar Bao604.

Sclerotinia phenotyping of DH lines: When the plants were at the flowering stage stems were inoculated at two internodes with plugs of sclerotinia mycelium as described in item 1. The length and texture of each lesion was recorded at weekly intervals for three weeks. DH lines were tested in groups of 30 with 4 plants per line and each line was tested twice. A third test was conducted of lines showing resistance or in cases when the phenotype of a line was in doubt. Reliable phenotypic disease data were obtained from 59 DH1-117 lines, 63 DH3 lines, 146 DH4 lines and 30 DH5 lines encompassing 28,800 data points (i.e. 300 lines x app. 8 plants/line x 2 inoculation sites/plant x 6 observations/inoculation site). Occasionally, lower than normal infection levels were recorded in tests involving lines of the DH1-117 and DH1-118 populations most likely as a result of larger stem diameter of these winter-type lines. Retesting of about half of each of the two DH1 populations is therefore needed.

There was a three to four fold difference between the disease reaction of the resistant parent, ZY821, and the susceptible parents. Average stem lesion length at 21 dai was 50 mm for ZY821 and

>100 mm for DHWestar and Bao 604; % soft + collapsed lesions was 25% for ZY 821 and >50% for the susceptible parents. The disease reaction of DH lines showed a continuous distribution typical of a quantitative host-pathogen interaction. Consequently, no clear cut-off between resistance and susceptible lines could be identified in any of the DH populations. DH lines with similar or more resistant phenotypes than ZY821 were identified. The number of resistant lines in each DH population indicated that three or more genes are necessary for resistance. Sclerotinia stem rot resistance was identified in fourteen DH lines, thereby demonstrating that this type of resistance is heritable and can be transferred to Canadian canola cultivars.

Marker screening: Five grams of leaf tissue was harvested from each of the 80 DH1-117 lines, 74 DH1-118 lines, 70 DH2 lines, 66 DH3 lines, 234 DH4 lines and 185 DH5 lines. The tissue samples were immediately placed in liquid nitrogen and thereafter freeze dried. Total genomic DNA was extracted from 0.5 g freeze dried tissue using Kirby's phenol/chloroform protocol.

Approximately 3000 microsatellite markers are available to researchers at AAFC for mapping of diverse traits in *B. napus* and other Brassica species. Around 700 markers were selected for mapping of sclerotinia resistance potentially amplifying about 900 loci at about 20 cM intervals along the 19 linkage groups of *B. napus*. Initially the parents, ZY821, Westar, DH6756 and Bao604, were screened with these markers (Table 1). Markers that were polymorphic between two parents were used to screen lines in each DH population and linkage analysis of those markers that were carried out with the software package Mapmaker. Of the 493 markers polymorphic between ZY821 and Bao 604 (Table 1) 192 and 189 loci could be mapped in the DH1-117 (Figure 2) and DH1-118 (Figure 3) populations, respectively. ZY821 and Westar had 455 polymorphic loci, of which 209 could be mapped in the DH3 population (Figure 4). In the backcross populations of the two most sclerotinia resistant lines to DH Westar, 94 loci could be mapped in DH4 population (Figure 5) and 83 loci could be mapped in the DH5 population (Figure 6). The reduced number of polymorphic loci in DH4 and DH5 was expected since Westar constituted a larger part of the genome and resulted in a number of monomorphic linkage groups N2, N4, N5 and N8 in DH4 (Figure 5); N7, N8 and N17 in DH5 (Figure 6). The polymorphism between the two parents ZY821 and DH6756 was extremely low (data not shown). The two lines were likely sister lines developed in the same breeding prog .m, which made it impossible to use the DH2 lines derived from this cross for mapping purposes.

Currently we are working to reduce the size of 35 marker intervals larger than 13 cM by screening additional microsatellite markers.

QTL analysis: The following five sclerotinia traits were used for QTL analysis; average lesion length of each line on three separate rating dates (7, 14 and 21 days after inoculation), area under the disease progress curve (AUDPC) using the average lesion length, and percent soft plus collapsed lesions (% s + c) over the three rating dates. The sclerotinia phenotyping data combined with results from screening of the microsatellite markers were analysed using QTL Mapmaker. Table 2 lists QTLs with LOD scores above 2.0 identified either using the entire DH1, DH3 and DH4 population of doubled haploid lines or only the most resistant and susceptible lines.

Most emphasis should be put on QTLs resulting from screening of the largest populations with the highest LOD score, and the shortest interval between the two markers for the QTL. Analysis

of the data from DH4 resulted in four QTLs on linkage groups N6, N7, N11 (LOD = 3.60, 1.3 cM) and N16 (LOD = 3.3, 16.8 cM). Screening of DH3 yielded six QTLs on linkage groups N3, N5 (LOD = 2.71, 2.9 cM), N16 and N19 (LOD = 2.78, 2.78 cM), and DH1-117 resulted in three QTLs on N9 (LOD = 2.66, 1.2 cM), N11 and N12, respectively. The variances explained by each sclerotinia trait were from 11.4 % to 19.4%. One or more of the five sclerotinia traits were associated with each QTL, but definite correlations were not observed. Higher LOD scores and increased % variance due to stem rot resistance can be expected after phenotyping of the remaining DH lines.

The lack of overlap among QTLs in the DH1 and DH3 derived populations may in part be due to different genetic contribution from the susceptible parent Bao 604 and DH Westar, the relative small population sizes, and the larger stem diameter of DH1 lines compared to lines in DH3, DH4 and DH5, which could have confounded the expression of disease symptoms. Two other research groups have published QTLs associated with sclerotinia resistance in canola. Osborn et al. (2005) identified QTLs on linkage groups N2, N5, N12 , N14, N16 and N19 all of which were identified in our project (Table 2). Zhao and Meng (2003) found QTLs on N7, N10 and N15. Primer information was not available for any of the published markers, so a direct comparison of data was not possible.

Table 1. Number of polymorphic loci by linkage group identified in five populations of doubled haploid *B. napus* populations and their respective parents.

Linkage group	Putative micro-satellite markers	Bao604 vs. ZY821	DH1-117 80 lines	DH1-118 74 lines	DHWestar vs. ZY821	DH3 63 lines	DHWestar vs. DH3-859	DH4 backcross 234 lines	DHWestar vs. DH3-869	DH5 backcross1 86 lines
N1	58	27	11	10	33	14	28	10	8	6
N2	41	13	8	7	23	10	1	0	3	3
N3	71	26	12	11	35	15	13	4	6	6
N4	34	22	5	5	18	9	0	0	3	3
N5	41	25	10	10	30	12	1	0	7	3
N6	62	40	11	11	27	12	16	7	5	4
N7	38	24	11	9	23	12	19	10	2	0
N8	39	21	8	7	20	9	0	0	0	0
N9	73	44	16	16	47	17	20	4	9	7
N10	60	42	13	11	40	11	38	8	11	8
AA genome	517	284	105	107	296	121	136	43	54	40
N11	54	11	8	7	21	10	21	9	7	7
N12	32	11	11	11	18	11	16	7	5	4
N13	87	42	15	15	36	16	10	4	7	6
N14	61	36	13	12	23	13	19	8	8	7
N15	47	27	9	9	3	2	4	2	2	2
N16	16	11	2	2	8	8	3	3	4	3
N17	42	21	9	6	13	8	10	4	1	0
N18	40	16	10	10	15	9	11	7	7	7
N19	50	34	10	10	22	11	11	7	7	7
CC genome	429	209	87	82	159	88	105	51	48	43
AACC genome	946	493	192	189	455	209	241	94	102	83

DH3-859 and DH#-869 are sclerotinia resistant spring type *B napus* lines

Figure 2. Linkage map of 192 microsatellite markers polymorphic in the DH1-117 population derived from Bao604 x Zhong You 821. Three putative QTLs conferring resistance to sclerotinia stem rot (grey boxes) were identified using phenotypic data from 59 DH lines.

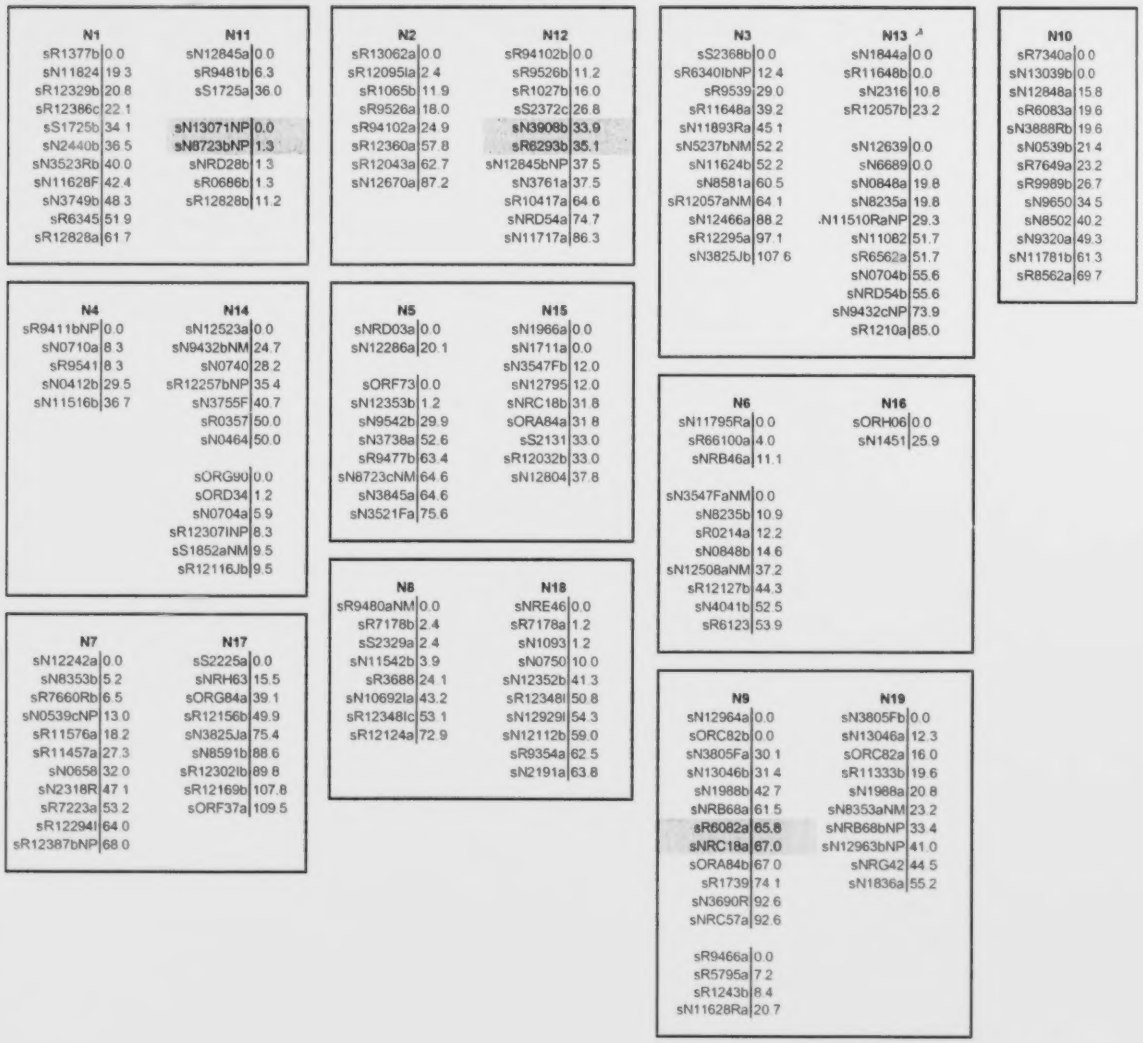

Figure 3. Linkage map of 189 microsatellite markers in the DH1-118 population derived from Bao604 x Zhong You 821. QTL analysis waiting for retesting of 74 DH lines for sclerotinia reaction.

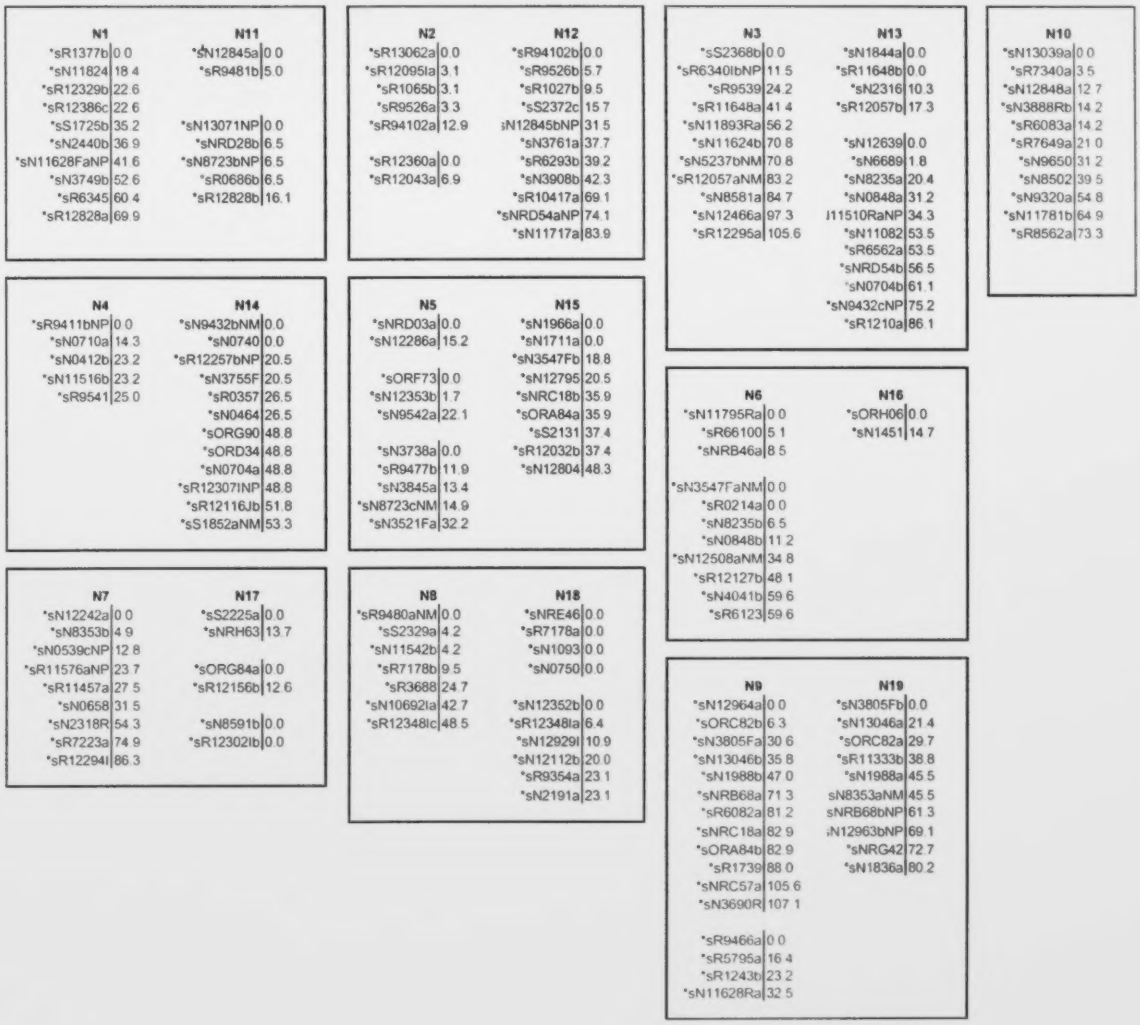

Figure 4. Linkage map of 204 polymorphic microsatellite markers the DH3 population derived from Westar x Zhong You 821. Five putative QTLs conferring resistance to Sclerotinia stem rot (grey boxes) were identified using phenotypic data from 63 DH lines.

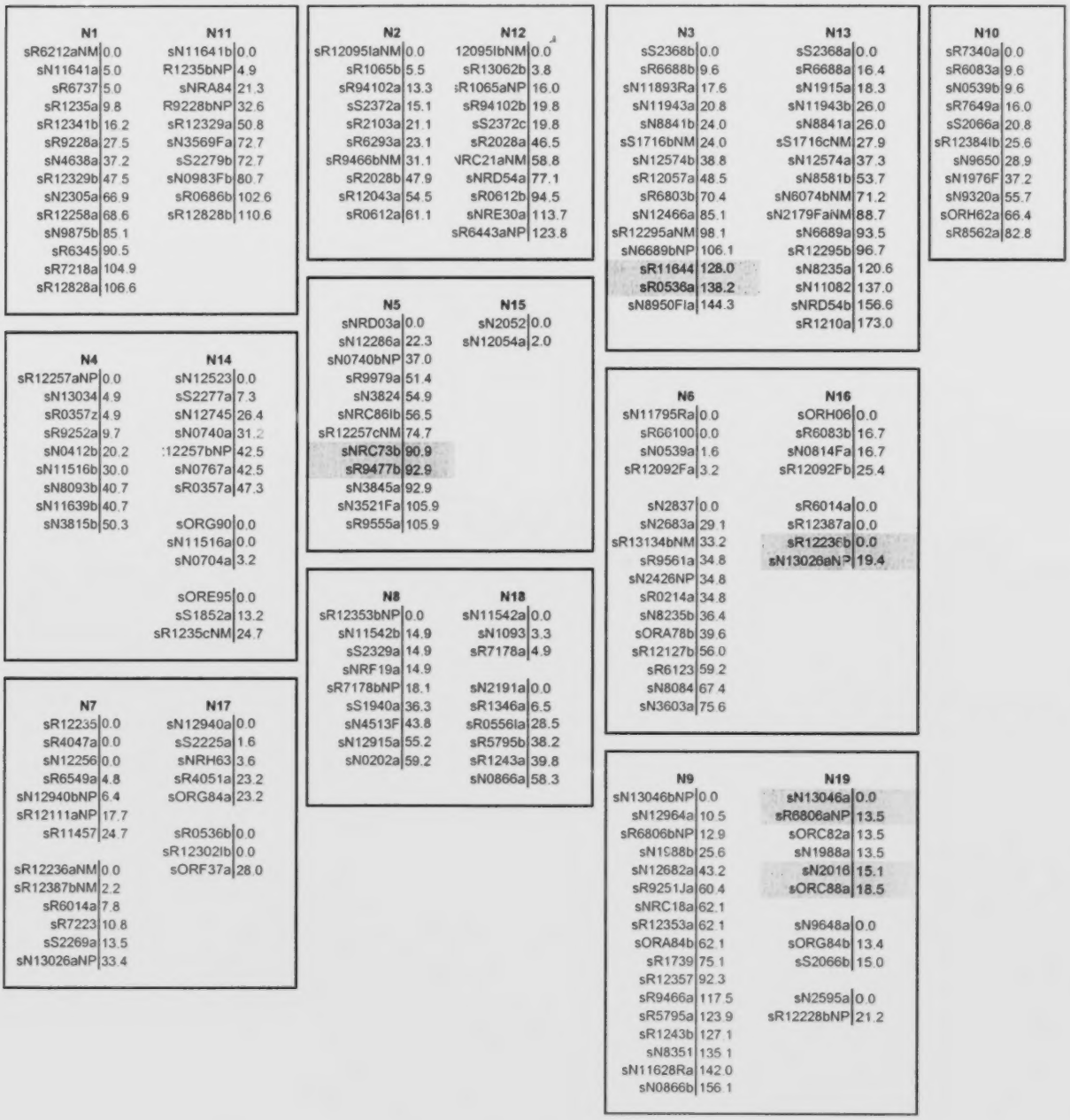

Figure 5. Linkage map of 93 polymorphic microsatellite markers in the DH4 population derived from a backcross DH Westar x DH3-59 (spring type with sclerotinia resistance). Four putative QTLs conferring resistance to Sclerotinia stem rot (grey boxes) were identified using phenotypic data from 146 DH lines.

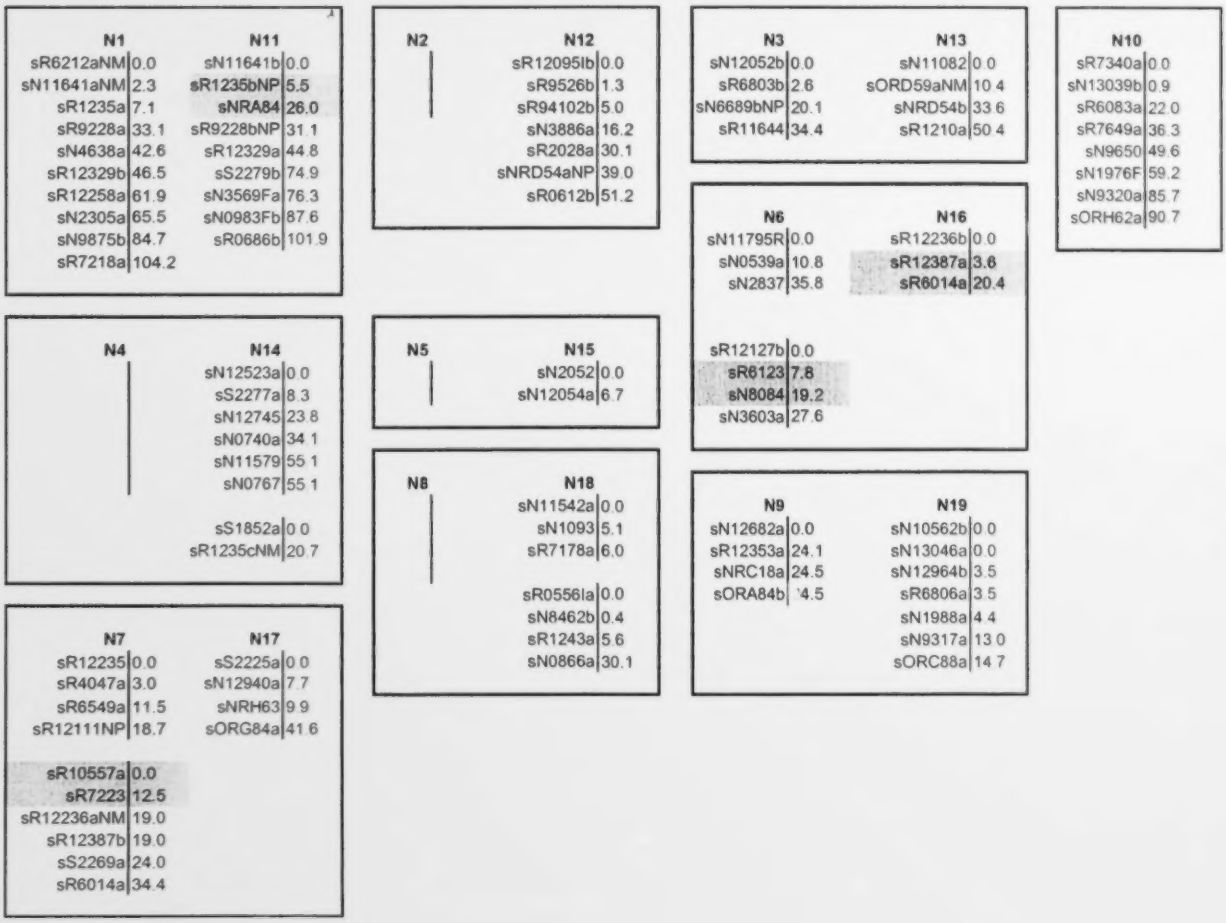

Figure 6. Linkage map of 83 polymorphic microsatellite markers in the DH5 population derived from backcross of DH Westar x DH3-69 (spring type with sclerotinia resistance). QTL analysis awaiting testing of 186 DH lines for sclerotinia reaction.

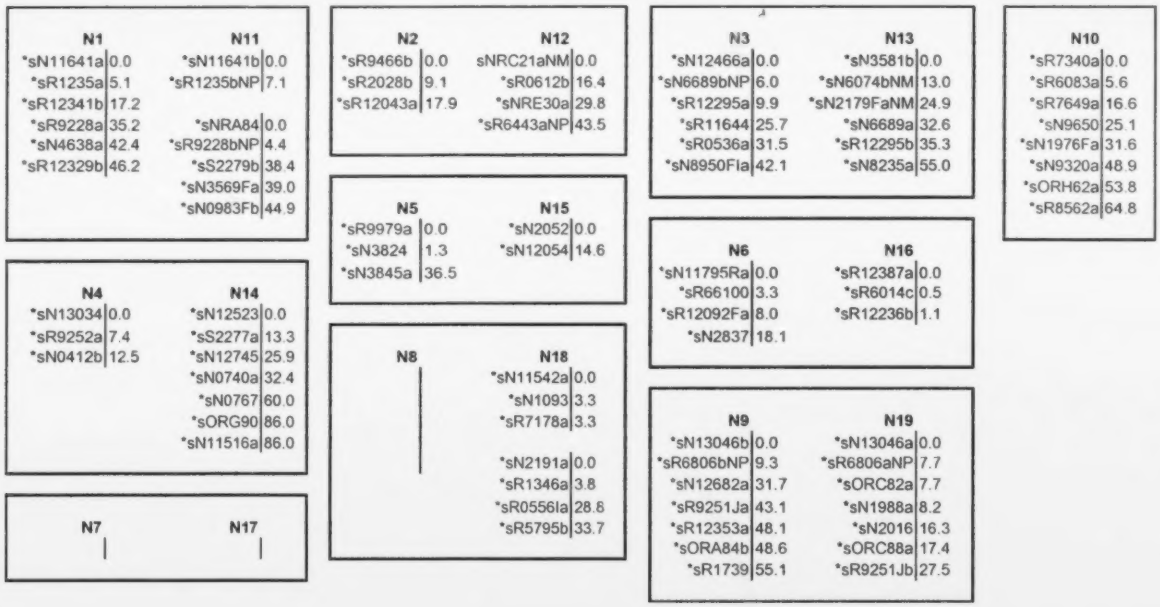

Table 2. Putative QTLs identified in three populations of doubled haploid lines conferring resistance to *Sclerotinia sclerotiorum*. The QTLs were identified either using the whole population of DH lines (grey boxes) or only the most resistant and susceptible (R + S) lines.

Linkage Group	Marker interval	Interval length	Doubled hapoid population or selected R and S lines	Sclerotinia trait	LOD score	% Variance
N2	sR12043a-sN12670a	24.5 cM	DH1-117 (10R + 11S)	21dai	4.19	91
				%s+c	6.16	94.2
N3	sR11644-sR0536a	10.2cM	DH3 (63 lines)	%s+c	2.00	13.6
N5	sNRC73b-sR9477b	2.9 cM	DH3 (63 lines)	7dai	2.71	18.0
			DH3 (4R + 11S)		2.45	53.3
			DH3 (63 lines)	14dai	2.18	14.8
				AUDPC	2.32	15.6
	sN3824-sR12257c	19.8 cM	DH3 (4R + 11S)	%s+c	6.57	97.0
N6	sN2837-sN2683a	29.1 cM	DH3 (4R + 11S)	14dai	2.98	87.3
				21dai	3.41	88.8
				AUDPC	4.10	91.0
	sORA78b-sR12127b	16.4 cM		%s+c	7.65	97.0
					6.51	97.0
	sR6123-sN8084	11.4 cM	DH4 (146 lines)	14dai	2.03	6.2
				21dai	2.16	7.2
				AUDPC	2.02	6.2
N7	sR10557-sR7223	12.5 cM	DH4 (146 lines)	%s+c	2.14	7.6
	sR11576a-sR11457a	9.1 cM	DH1-117 (10R + 11S)	21dai	2.15	38.8
				%s+c	2.73	45.1
N9	sR6082a-sNRC18a	1.2cM	DH1-117 (59 lines)	21dai	2.66	18.8
			DH1-117 (10R + 11S)	7dai	2.05	36.1
				14dai	2.14	37.5
				21dai	2.05	36.2
				AUDPC	2.20	38.2
N11	sR1235bN-sNRA84		DH4 (146 lines)	%s+c	3.60	11.4
	sN13071NP-sN8723bNP	1.3 cM	DH1-117 (59 lines)	21dai	2.16	15.6
				AUDPC	2.10	14.9
				%s+c	2.24	16.1
			DH1-117 (10R + 11S)	14dai	2.03	35.9
				AUDPC	2.13	37.3
N11 (2 peaks)				21dai	2.29	39.5
				%s+c	2.36	40.4
N12	sN3908b-sR6293b	1.2 cM	DH1-117 (59 lines)	7dai	2.10	15.1
N14	sN12523a-sN9432bNM	24.7 cM	DH1-117 (10R + 11S)	21dai	2.05	55.7
N16	sR12387a-sR6014c	16.8 cM	DH4 (146 lines)	7dai	3.30	11.5
					2.99	80.7
	sR12236b-sN13026a	19.4 cM	DH3 (4R + 11S)	14dai	4.34	87.3
				21dai	4.77	88.8
				AUDPC	5.46	91.0
			DH3 (63 lines)	%s+c	2.04	14.0
			DH3 (4R + 11S)		9.01	97.0
N18	sR1346a-sR0556la	22 cM	DH3 (4R + 11S)	AUDPC	2.97	91.0
N19	sN2016-sORC88a	3.4 cM	DH3 (63 lines)	21dai	2.78	19.4
			DH3 (4R + 11S)		2.78	59.9
				AUDPC	2.01	48.1
	sN13046aNM-sR6806aNP	13.5 cM	DH3 (63 lines)	%s+c	2.04	15.6

Examination of dominance of sclerotinia resistance and effect of individual QTLs

Reciprocal crosses were made among resistant (R) and moderately resistant (MR) doubled haploid lines identified during sclerotinia phenotyping of the DH1 and DH3 populations. Two of the resistant DH lines, DH3-59 and DH3-69, were also backcrossed to both the resistant parent ZY821 and the susceptible parent DH Westar (Table 3).

Sclerotinia phenotyping of F_1 plants from twelve reciprocal crosses (R x R and R x MR) and the two backcrosses were carried out using the method described in item 1. F_1 derived from the backcrosses to the susceptible parent were as susceptible as DH Westar showing that stem rot resistance is inherited in a recessive manner. F_1 plants derived from R x R and R x MR crosses varied from highly resistant to almost as susceptible as DH Westar. Assuming stem rot resistance is recessive, the most resistant F_1 plants were likely derived from crosses between two DH lines sharing QTLs most responsible for the resistance expression. Currently, there are too may putative QTLs with LOD scores around 2.0 (Table 2) to allow conclusions on the effect of each individual QTL.

Table 3. Crosses made among DH lines resistant or moderately resistant to sclerotinia stem rot to examine the contribution of different QTLs to resistance by inoculation of F_1 plants with sclerotinia.

Female		Male	F1 seeds	Reciprocal F1 seeds	Total F1 seeds
64	x	16	10	46	56
	x	95	70	50	120
	x	13	22	28	50
	x	59	0	34	34
	x	63	2	0	2
	x	69	0	117	117
	x	89	0	161	161
95	x	63	200	145	345
	x	89	10	125	135
16	x	13	24	60	84
	x	59	2	51	53
	x	63	43	101	144
	x	69	9	25	34
	x	89	99	54	154
13	x	59	15	41	55
	x	63	13	39	52
	x	69	2	39	40
	x	89	4	0	4
59	x	63	10	1	10
	x	69	46	75	121
	x	89	308	0	308
63	x	69	8	15	23
	x	89	3	100	103

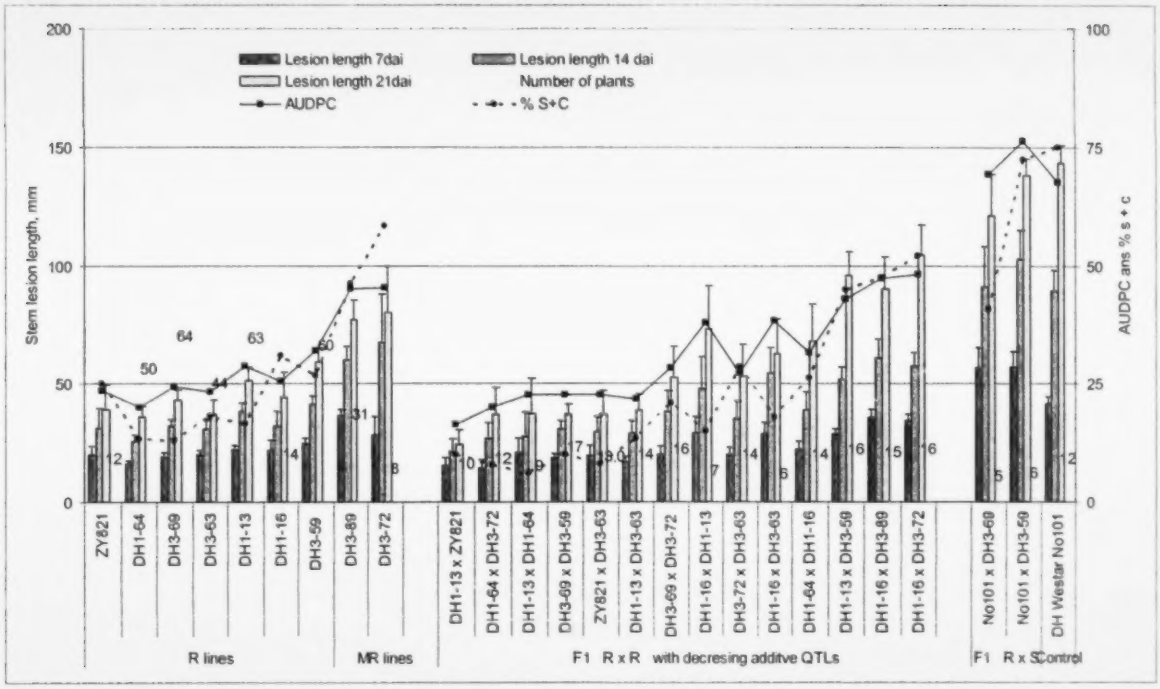

Figure 7. Reaction of F_1 plants derived from crosses among resistant (R) and moderately resistant (MR) doubled haploid lines, including Zhong You 821, showing an additive effect of some of the QTLs contributing to sclerotinia resistance. The susceptible disease reaction of F_1 derived from crosses of two resistant lines DH3-59 and DH3-69 with the susceptible Westar No101 shows that resistance to sclerotinia is recessive.

Publications

Buchwaldt, L., Hegedus, D, Li, R., **Lydiate, D**, and **Rimmer, R.** 2005. Preliminary mapping of physiological resistance to *Sclerotinia sclerotiorum* in *Brassica napus*. The 13th International Sclerotinia Workshop, June 12-16, 2005, Monterey, CA, USA.

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Hegedus, D., **Buchwaldt, L.**, Li, **R., Rimmer**, R. and Sharpe, A., 2004. Gene Expression Associated with Sclerotinia Infection and Resistance in *Brassica napus*. Canadian Crop Genomics Initiative Annual Meeting. Ottawa (ON), June.

Hegedus, D., **Buchwaldt, L.**, Li, R., **Rimmer, R**. and Sharpe, A., 2004. Gene Expression Associated with Sclerotinia Infection and Resistance in *Brassica napus*. Canadian Crop Genomics Workshop. Quebec City (QB), August.

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Rimmer, R., Buchwaldt, L., Séguin-Swartz, G., Boyetchko, S., Li, R., Brkic, M. and Hegedus, D., 2003. Developing and engineering resistance to sclerotinia stem rot in canola. Sclerotinia Workshop at the 8th International Congress of Plant Pathology, Christchurch, New Zealand, February 2-7.

(d) Project Developed Materials

1) A total of 14 doubled haploid *B. napus* lines with resistance to sclerotinia stem rot have been identified so far; 3 spring type DH lines will be included in the Plant Gene Resources of Canada collection.

2) One spring-type DH line with high level of sclerotinia stem rot resistance was identified and transferred to the AAFC canola breeding program (Drs. G. Seguin-Swartz and G. Racow). Resistance is being incorporated into a yellow-seeded, canola quality cultivar using traditional plant breeding techniques. The F_4 have low erucic acid levels, but variable levels of glucosinolate and needs further selection.

3) Two large sclerotinia mapping populations of doubled haploid lines were developed of *Brassica napus* and seeds were obtained from 419 DH lines. These lines will contribute to fine-mapping of sclerotinia resistance.

4) Linkage maps of the 19 *B. napus* chromosomes with QTLs conferring sclerotinia resistance have been developed.

5) In a related project 5 resistant and 5 susceptible DH lines enabled identification of the first set of *B. napus* genes induced in the defence response to sclerotinia infection (Drs. Hegedus, Rimmer and Buchwaldt).